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# Gene conversion plays the major role in controlling the stability of large tandem repeats in yeast

**Serge Gangloff, Hui Zou and Rodney Rothstein[1]**

Department of Genetics and Development, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, NY 10032, USA

S.Gangloff and H.Zou contributed equally to the work reported here

[1]Corresponding author

**The genomic stability of the rDNA tandem array in yeast is tightly controlled to allow sequence homogenization and at the same time prevent deleterious rearrangements. In our study, we show that gene conversion, and not unequal sister chromatid exchange, is the predominant recombination mechanism regulating the expansion and contraction of the rDNA array. Furthermore, we found that *RAD52*, which is essential for gene conversion, is required for marker duplication stimulated in the absence of the two yeast type I topoisomerases. Our results have implications for the mechanisms regulating genomic stability of repetitive sequence families found in all eukaryotes.**

*Keywords*: gene conversion/rDNA/*Saccharomyces cerevisiae*/topoisomerases

## Introduction

Genetic recombination in repetitive DNA is thought to be the major mechanism governing the evolution of multigene families, as well as alterations of genome structure (Edelman and Gally, 1970). In the yeast *Saccharomyces cerevisiae*, the rDNA constitutes a model system for studying the genetic control of repetitive sequence stability. It is organized as a single cluster of ~100–200 tandem repeats of 9.2 kb on chromosome XII (Petes, 1979). Due to its importance in ribosome biogenesis, the nucleotide sequence of this cluster needs to be maintained with a high degree of fidelity. A tight control mechanism must be in place to balance the propensity toward high levels of recombination in directly repeated sequences, which can potentially lead to loss of information (Jackson and Fink, 1981; Klein and Petes, 1981; Klein, 1984; Willis and Klein, 1987), with the need for sufficient recombination to homogenize the sequences in the array. Recently, it has been found that mutations in either of the genes encoding the type I topoisomerases (*TOP1* or *TOP3*) result in increased mitotic instability in yeast rDNA (Christman *et al.*, 1988; Gangloff *et al.*, 1994a).

Several mechanisms have been proposed to explain the rearrangements observed in the rDNA array (reviewed in Gangloff *et al.*, 1994b) (Figure 1). Intrachromatid recombination and unequal sister chromatid exchange (USCE) can be detected molecularly, since they are accompanied by the formation of well-defined reciprocal products, a circle and a duplication, respectively (Larionov *et al.*, 1980; Petes, 1980; Szostak and Wu, 1980). On the other hand, processes like gene conversion (GC) or single strand annealing (SSA) can be distinguished through their differential requirement for the evolutionarily conserved gene, *RAD52* (Resnick, 1969; Adzuma *et al.*, 1984; Bezzubova *et al.*, 1993; Ostermann *et al.*, 1993; Bendixen *et al.*, 1994; Muris *et al.*, 1994). In yeast, *RAD52* is involved in the repair of DNA double-strand breaks (DSBs) (for review, see Petes *et al.*, 1991), is necessary for gene conversion (Petes *et al.*, 1991) but is not required for the generation of marker loss by SSA in the rDNA (Ozenberger and Roeder, 1991).

It is widely believed that USCE is the major mitotic event responsible for controlling the size and homogeneity of repetitive sequences (Szostak and Wu, 1980). If this process were operating efficiently on all repetitive sequences, many deleterious deletions, inversions or modifications in gene dosage would arise (Jackson and Fink, 1981; Klein and Petes, 1981). Nevertheless, USCE is often invoked by researchers to explain many rearrangements in higher eukaryotic cells (Lehrman *et al.*, 1987; Oberle *et al.*, 1991; Warburton *et al.*, 1993). Furthermore, the rapid evolutionary changes observed in multiple tandem arrays cannot always be explained simply by USCE, which is a relatively slow process (Roberts and Axel, 1982; Charlesworth *et al.*, 1994). In many cases, it seems more likely that gene conversion is responsible for the rapid spread of information.

In this report, we use the yeast rDNA model system to demonstrate that gene conversion and not USCE plays the major active role in controlling copy number and sequence homogeneity in multiple repeats. We also show that the two known yeast type I DNA topoisomerases exhibit different functions that together prevent structures resulting from transcription and/or replication from being resolved as recombinants. These results have direct implications for the mechanisms controlling stability of repetitive sequence families found in all eukaryotes.

## Results

### *USCE is not the major event leading to marker loss in wild-type yeast*

We and others have found that the absence of either eukaryotic type I DNA topoisomerase (Top1 or Top3) results in increased recombination in the yeast rDNA multiple tandem array (Christman *et al.*, 1988; Gangloff *et al.*, 1994a). During the investigation of the mechanisms responsible for this increased recombination, we first examined the kind of events occurring in our wild-type strain as a control. Previously, it has been reported that USCE is the predominant event leading to marker loss in

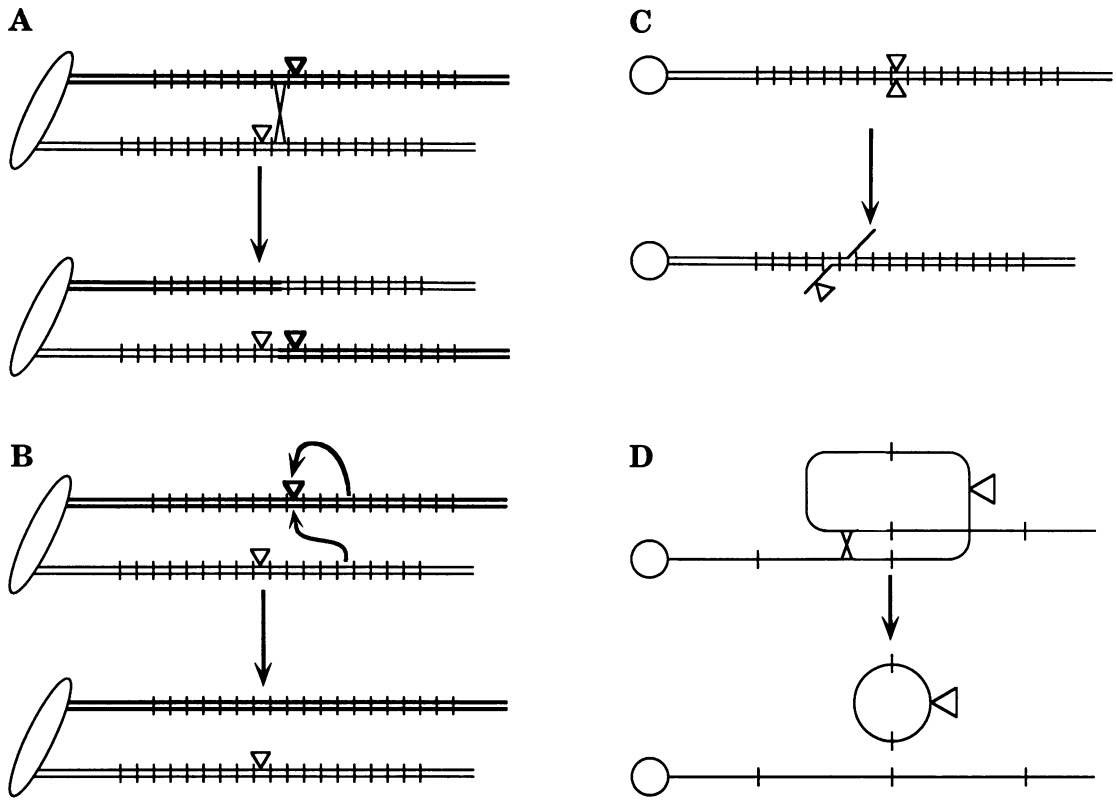


**Fig. 1.** Potential recombination mechanisms for marker loss in a multiple tandem array. (**A**) Unequal sister chromatid exchange occurs when misaligned repeats recombine reciprocally. If this event occurs between the inserted marker (as depicted in the figure), two non-identical sisters are generated in the following mitosis: one exhibiting marker loss and the other containing two copies of the insert separated by the number of repeats that were misaligned. (**B**) Gene conversion can take place in a multiple tandem array at either $G_1$ or $G_2$. The direction of the non-reciprocal transfer of information is depicted by the arrows. (**C**) A double-strand break in a multiple tandem array can be repaired using a single-strand annealing mechanism. Nucleases attack the exposed ends, probably degrading from the 5′ end. When homologous regions between adjacent repeats are revealed, the single strands can anneal to create a repairable structure that leads to an intact array. If the break occurs near the repeat containing the inserted marker, the marker may be lost during the repair of the break as illustrated in the figure. (**D**) Ring formation can occur when adjacent repeats pair and reciprocally recombine. The size of the ring varies as a function of the number of copies of the repeat between the paired substrates. In the example illustrated, the crossover takes place with an extra repeat between the paired substrates, giving rise to a dimer ring. If the event includes the inserted marker, then the marker is incorporated onto the ring. Note that, in this panel, the single line represents both strands of the duplex.

rDNA (Szostak and Wu, 1980; Zamb and Petes, 1981). We investigated the occurrence of this event in half-sectored colonies. We modified the strategy developed by Szostak and Wu (1980) to allow the resolution of larger DNA restriction fragments (see Figure 2A). Half-sectored colonies are a consequence of marker loss in one of the sister cells during the first mitotic division after plating. If marker loss is generated by classical USCE, the marker will be duplicated in the population of cells derived from the sister, and the distances between the insert and the borders of the array will remain unchanged (Figure 2A, fragments L and R).

In W303 derivatives, we found that only three half-sectored colonies out of 21 analyzed (14%) displayed marker duplications and that the duplications were separated by 1–10 rDNA repeats according to the nomenclature of Szostak and Wu (1980) (data not shown). We could not measure small variations in fragments L or R due to their large size. Since Szostak and Wu (1980) reported that six out of seven sectored colonies that they characterized showed duplications (86%), we determined whether this discrepancy is due to differences in strain backgrounds. We isolated and analyzed 10 half-sectored colonies in the T16 strain used in their report. Figure 2B and C shows the results of hybridization of a genomic blot of *Pst*I-digested chromosomal DNA from five such half-sectors. The *Pst*I restriction enzyme cuts once in the *LEU2* marker embedded in rDNA and also in sequences outside of the array. In Figure 2B, the filter was hybridized with a probe that detects only one of the two fragments (R described in Figure 2A) between the *Pst*I site within the insert and the first *Pst*I site flanking the array. This probe can also identify a fragment that results from marker duplication (Figure 2A, fragment I). Only one of the half-sectors shown in Figure 2B exhibits marker duplication (lane 1) since two fragments were detected. In total, three out of the 10 half-sectored colonies analyzed revealed a marker duplication (data not shown). This frequency (30%) is not statistically different from that observed in the W303 background (14%, $P = 0.6$).

The same filter shown in Figure 2B was re-hybridized with a probe that detects all of the rDNA restriction fragments (Figure 2C). The additional band detected in each lane corresponds to the fragment between the *Pst*I site in the marker and the *Pst*I site on the other side of the rDNA array (fragment L). This fragment can be resolved on the gel; however, the position of the *Pst*I site in the non-rDNA sequences is unknown. Thus, we can

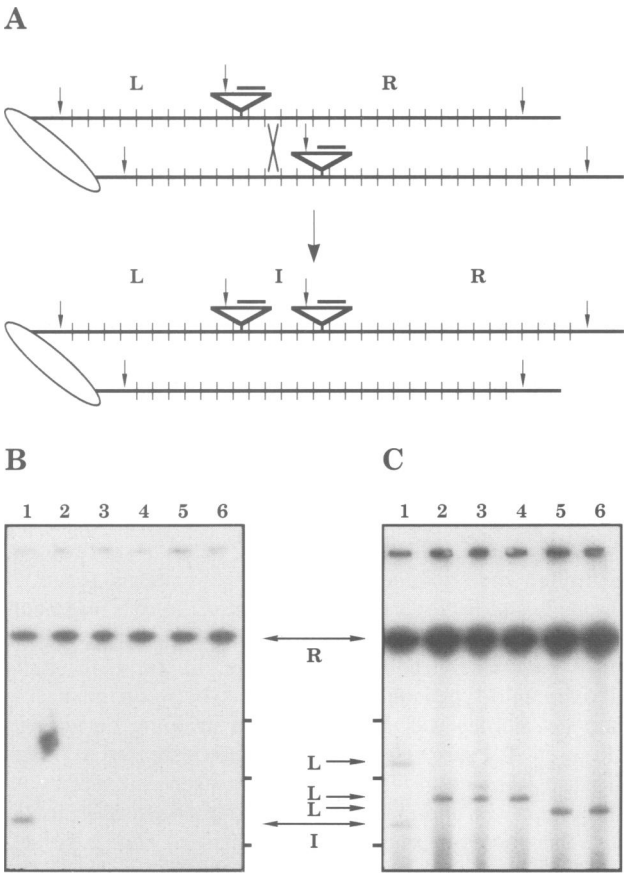


**Fig. 2.** Analysis of USCE in the T16 strain. (**A**) Generation of a half-sectored colony by USCE. A reciprocal exchange between misaligned repeats on sister chromatids in $G_2$ leads to a duplication of the marker (inverted triangle) on one chromatid associated with a loss on the sister chromatid. After mitosis, only half the cells in the colony contain the marker. These events are detected as sectored colonies after replica plating on omission medium. Digestion of genomic DNA with restriction enzymes (arrows) that cleave once in the marker but not in rDNA (*SalI* for *URA3* and *HIS3*, and *PstI* for *LEU2* markers), yields the L and R parental bands. If USCE occurs, it generates an additional band I corresponding to the total length of the inserted marker plus a discrete number of rDNA repeats. In addition, the lengths of both fragments L and R remain unchanged. The restriction fragments can be analyzed after separation by CHEF electrophoresis. (**B**) Chromosomal DNA from the marker-containing side of half-sectored colonies (lanes 1–5) was digested with *PstI*. Lane 6 contains DNA isolated from the parental T16 strain. The fragments were separated by CHEF electrophoresis using a pulse time ramping from 0.2 to 13 s for 15 h at 200 V, a condition that allows the resolution of fragments sizes ranging from 6 to 200 kb. The DNA was transferred to nylon and hybridized with the 980 bp *PstI–NheI* fragment of pBR322 [illustrated by the line on the top of the triangle in (A)], which detects fragments I and R. The size of the fragments was determined by comparison with Low Range PFG Markers from New England BioLabs shown as bars on the side of the gel (48.5, 97 and 145 kb). (**C**) The same blot as in (B) was probed with total labeled pBR322 to detect all fragments (L, I and R).

estimate that the *LEU2* marker is located at most eight rDNA units away from the border of the array (~75 kb fragment/9.2 kb rDNA unit size ≈8 units). This observation may account for the misinterpretation made earlier (Szostak and Wu, 1980), which explained the 50–70 kb signals detected on the autoradiograms as marker duplications separated by 6–8 repeats, instead of merely the distance of the marker to the non-rDNA junction.

In T16, since the distance between the inserted marker and one edge of the array is small (~75 kb), it is therefore possible to detect any variation in the number of rDNA repeats between the insertion and the edge of the array (fragment L). We found that the fragment L unexpectedly changed in four of the 10 half-sectors analyzed. All four of these are shown in Figure 2C (lanes 1–4). The simultaneous change in fragment L accompanied by a marker duplication (Figure 2C, lane 1) is in contradiction to classical USCE, since this process does not involve any change in the parental fragments L and R.

### *Gene conversion accounts for the change in the distance between the marker and the border of the array*

There are two possible explanations for the variation in the size of fragment L accompanying marker loss: either the variation and the marker loss are the result of a concerted event or the altered fragment L configuration reflects a pre-existing recombination event that occurred before the cells were plated. To distinguish between these possibilities, we designed an assay that permits the simultaneous analysis of a sectored colony along with the parental configuration from which it arose. We analyzed colonies in which marker loss took place during the second division on the plate. At this four-cell stage, two cells retaining the parental configuration are adjacent to the sister cells that underwent recombination (Figure 3A). When the resulting colony is replica plated onto omission medium, a quarter-sectored colony is revealed in which the non-growing sector is flanked by parental cells on one side and sister cells on the other. We can determine whether the change in fragment L was due to a pre-existing recombination event or was generated during the marker loss event itself by analyzing the marker configuration in the two flanking quarter-sectors. If the sizes of fragment L in both of the quarter-sectors are different from the parental configuration, then the alteration was due to a pre-existing recombination event. On the other hand, if a change in the size of fragment L is detected in only one of the quarter-sectors, then cells in that quarter-sector underwent the alteration of fragment L in concert with the marker loss.

We analyzed 11 independently isolated quarter-sectored colonies from T16 and none showed any pre-existing change in the parental configuration of fragment L, suggesting that these kinds of events are infrequent (eight are shown in Figure 3B). As observed during the analysis of half-sectors (Figure 2C, lanes 2–4), one of the quarter-sectored events exhibited a change in fragment L with no associated duplication (Figure 3B, pair 3). This rearrangement is most easily explained by a gene conversion event resolved by a cross-over between misaligned sister chromatids (unequal sister chromatid gene conversion, USCGC, see Figure 6). Four of the 11 quarter-sectors resembled classical USCE, in that fragment L is unchanged in the sister sector containing the marker duplication (two are shown in Figure 3B, pairs 2 and 6). However, they may actually be the result of gene conversion events associated with a change in length of fragment R, which we would not be able to detect. Finally, we observed another case similar to the half-sector described above (Figure 2C, lane 1) where a marker duplication in the

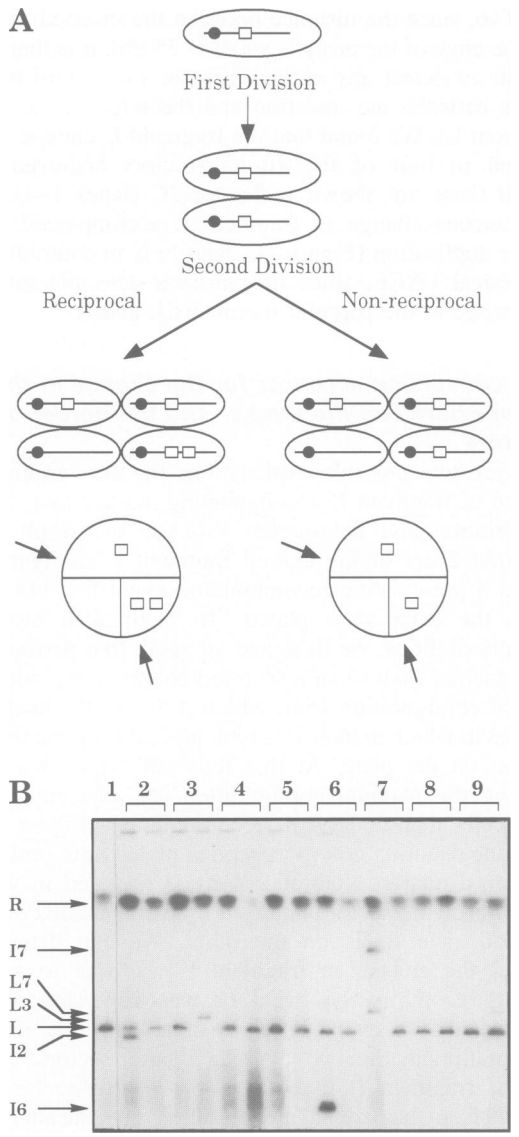


**Fig. 3.** Quarter-sector analysis of the T16 strain. (**A**) The pattern of the first two cell divisions after plating is outlined. The centromere of chromosome XII (•) and the marker inserted in rDNA (□) are shown. The recombination event of interest occurs in the lower cell during the second division. Two outcomes are illustrated: on the left, a quarter-sector resulting from USCE and on the right, a quarter-sector resulting from non-reciprocal marker loss. Below each quartet of cells, a quarter-sectored colony is depicted. The marker content of the two quarter-sectors (indicated by arrows) adjacent to the non-growing quarter-sector was measured. This determines both the parental marker configuration and the configuration after the recombination event. (**B**) Pairs of quarter-sectors marked by the arrows in (A) were analyzed as described in Figure 2C. L, I and R are the same as defined in Figure 2A and the numbers associated with L or I indicate the corresponding pairs (2, 3, 6 or 7) where the fragments differ from the parental configuration. L and I were assigned based on an additional genomic blot probed with a 980 bp *PstI–NheI* fragment of pBR322 as described in Figure 2B (data not shown).

sister is accompanied by the simultaneous lengthening of fragment L (Figure 3B, pair 7).

### *rDNA repeats are preferentially lost in rad52 mutants*

*RAD52* is essential for gene conversion (reviewed in Petes *et al.*, 1991), which can allow both the expansion and contraction of a multiple tandem array. To test the idea that the spontaneous rearrangements occurring in rDNA are due to gene conversion, we measured the length of the array in both wild-type and *rad52* mutants. We expect that, during vegetative growth in wild-type cells, the array can expand and contract spontaneously. In *rad52* mutants, where gene conversion is blocked, the array will only contract via a non-conservative mechanism similar to that found when induced lesions were examined (Ozenberger and Roeder, 1991).

To investigate changes in the length of the rDNA array within a given genotype, the distance between the marker and adjacent non-rDNA sequences (e.g. fragments L and R in Figure 2A) was compared between a parental spore clone and 12 clonally derived colonies that were separated by 30 generations from the parent. Both wild-type and *rad52* segregants from the same cross were compared to ensure that the rDNA configuration in the starting material was equivalent. Wild-type and *rad52* parental spore clones exhibited indistinguishable restriction patterns for fragments L and R (Figure 4). Only one of the two fragments could be measured accurately on this gel (L), since the other runs in the compression zone. In the 12 clonally derived wild-type isolates, the distance between the marker and the border was both increased in some derivatives (Figure 4A, lanes 3, 6, 11 and 13) and decreased in others (Figure 4A, lanes 4, 7, 8, 10 and 12) when compared with the parent. In contrast, the distance between the marker and the border in the 12 clonally derived *rad52* isolates is either identical to or smaller than the distance found in the parent spore clone. Additionally, in one *rad52* isolate, the 'unresolvable' fragment R has lost so many repeats that it can now be resolved on this gel (Figure 4B, lane 2). These results show that within 30 generations only contraction is observed in *rad52* mutants, where it is thought that a non-conservative process, like SSA, repairs spontaneous lesions in rDNA when the *RAD52* pathway is blocked. On the other hand, both expansion and contraction of the distance between the marker and border of the array is observed in *RAD52* strains, supporting the notion that these rapid changes are due to gene conversion.

### *RAD52-dependent duplications are elevated in the absence of type I DNA topoisomerases*

The two yeast type I topoisomerases are required for genomic stability of the rDNA multiple tandem array. Previous reports suggested that the absence of *TOP1* results in a 25-fold increase in marker loss in *top1* cells (Levin *et al.*, 1993; R.Keil, personal communication). The absence of the other type I topoisomerase from yeast, *TOP3*, causes a 75-fold increase in marker loss (Gangloff *et al.*, 1994a). The increased recombination in these topoisomerase mutants facilitates the examination of a large number of recombinants and provides a tool to explore whether the elevated recombination is also due to gene conversion. Therefore, we measured recombination frequencies and analyzed rearrangements in these two topoisomerase mutants in both the presence and absence of *RAD52*—these topoisomerase mutants facilitate the molecular analysis of a greater number of recombinants. To explore further how these two genes help to maintain genomic stability between the rDNA repeats, we measured recombination frequencies and analyzed molecularly the

events resulting from the absence of these two topoisomerases.

Table I shows the results of analyzing the various mutant combinations. First, we observed that two independent markers inserted in the rDNA array are lost at the same frequency ($1.3\times10^{-3}$ for *URA3* and $1.4\times10^{-3}$ for *HIS3*), indicating that the type of marker has little or no effect on the frequency of its loss. The *top1 top3* double mutants display a 216-fold increase in marker loss, indicating a synergistic interaction. In the absence of *RAD52*, marker loss is paradoxically elevated 7- to 10-fold. The elevated recombination observed in *top1 rad52* double mutants (29-fold) is not significantly different from that observed in *top1* strains (31-fold), indicating that some portion of the elevated recombination in *top1* mutants is *RAD52* independent. On the other hand, the *top3* elevated recombination is entirely dependent upon *RAD52*. These results suggest that the lesions that accumulate in the absence of these type I topoisomerases are processed differently.

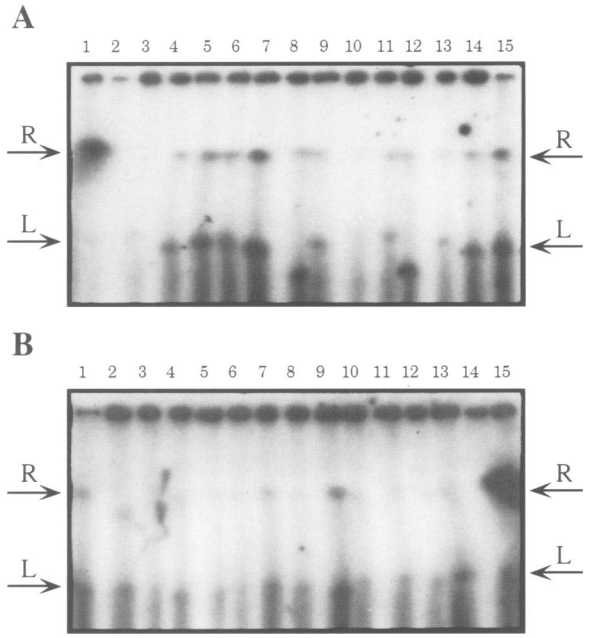


**Fig. 4.** Shrinkage of the rDNA array in *rad52* mutants. The distance between the *URA3* marker and non-rDNA sequences (fragment L) was compared in *RAD52* wild-type and *rad52* mutant strains. For each strain, agarose blocks were made from one spore clone and 12 clonally derived colonies separated from it by 30 generations. To minimize any possible difference in the rDNA configuration between the starting clones, the wild-type and *rad52* spores were segregants from the same cross. Unless noted, DNA was digested with *SalI* and separated by CHEF electrophoresis (190 V, 15 h with a 60 s pulse plus 15 h with a 90 s pulse). After transfer, the membrane was hybridized with the PCR-generated rDNA probe. (**A**) *RAD52* cells: lane 1, undigested DNA from the spore clone; lanes 2 and 15, DNA from the spore clone; lanes 3–14, DNA from 12 clonally derived colonies. (**B**) *rad52* cells: lanes 1 and 14, DNA from the spore clone; lanes 2–13, DNA from 12 clonally derived colonies; lane 15, undigested DNA from the spore clone. The letters L and R are defined in Figure 2A. Note, the lanes at the edge of this CHEF gel are not perfectly straight. The L band seen in lane 1 of (A) is really from lane 2. Similarly, the L band seen in lane 15 of (B) is from lane 14. The lengths of parental fragments R and L are ~1.3 and 1.0 Mb, respectively. Some of the background bands in this figure are probably due to secondary recombination events that occur during growth of the colony in preparation for analysis.

We next examined the *RAD52* dependence of marker duplication in the topoisomerase mutant backgrounds beginning with *top1*. To ensure that the starting configuration of the markers in the rDNA array was identical, *top1* and *top1 rad52* segregants came from the same cross. When sectored colonies generated in *top1* mutant cells were examined, marker duplications were found in almost every half-sector analyzed (16 out of 22). The major class of duplications is always the one where the markers are separated by a single rDNA repeat (see Figure 5A). In all of the colonies analyzed, including those that did not contain a major duplication, faint signals are observed. These can be explained as duplications that were generated later during colony growth and therefore are not primary events. Since both USCE and gene conversion can potentially lead to the marker duplication observed, we investigated whether these duplications are always associated with marker loss, which is the hallmark of USCE. We therefore analyzed random unsectored colonies from the same plates where the half-sectors were picked. In two out of six colonies tested (Figure 5A, lanes 5 and 6), the pattern of marker duplications was identical in size and intensity to that seen when half-sectored colonies were analyzed. We demonstrated that this pattern was due to a single duplication in most of the cells in the colony and not the consequence of multiple tandem duplications in a few cells (Figure 5B). We conclude that marker duplication can occur independently of marker loss. It is likely that such duplications arise mainly through a non-reciprocal event like gene conversion.

In *top1 rad52* double mutants, only 1/16 of the sectored colonies exhibit a marker duplication (see Figure 5D, lane 7). This ratio is similar to that observed in *rad52* single mutants (1/12, data not shown). In addition, no duplications

**Table I.** Mitotic frequency of marker loss in the rDNA

| Genotype | rDNA::*URA3* | | rDNA::*HIS3* | |
|---|---|---|---|---|
| | Recombination frequency $\times10^{-3}$ | Fold increase | Recombination frequency $\times10^{-3}$ | Fold increase |
| Wild-type | 1.3 ± 0.3 | 1 | 1.4 ± 0.6 | 1 |
| *top1* | 31 ± 5 | 25 | 45 ± 10 | 31 |
| *top3* | 96 ± 15 | 76 | 117 ± 15 | 81 |
| *rad52* | 9 ± 5 | 7 | 14 ± 4 | 10 |
| *top1 top3* | 281 ± 115 | 216 | ND | ND |
| *top1 rad52* | ND | ND | 41 ± 6 | 29 |
| *top3 rad52* | ND | ND | 17 ± 2 | 12 |

The recombination frequencies were determined as described in Materials and methods. The values reported are the means and standard deviations determined on at least six independent trials for each genotype. ND: not determined.

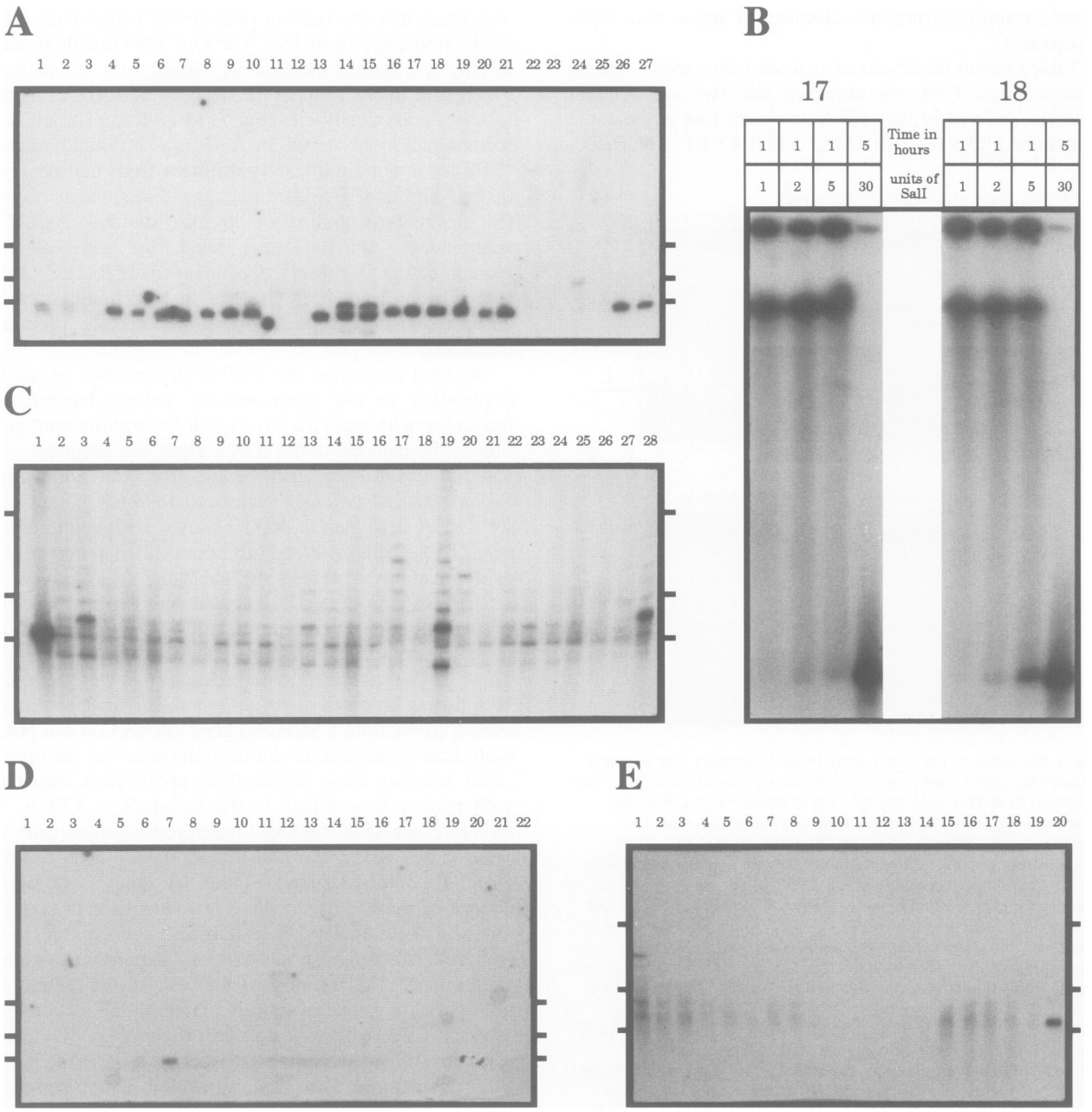


**Fig. 5.** Marker duplications in topoisomerase mutants and how they are affected by mutations in *RAD52*. (**A**) DNA from unsectored (lanes 1–6) and sectored (lanes 7–27) *top1-8*::*LEU2* rDNA::*HIS3* colonies was isolated, digested with *Sal*I and separated by CHEF electrophoresis (pulse time ramping from 1 to 50 s for 20 h at 200 V). The gel was blotted to a nylon membrane and hybridized with $^{32}$P-labeled pUC18 DNA. The size of the fragments was determined by comparison with Low Range PFG Markers from New England BioLabs shown as bars on the side of the gel (23.1, 48.5, and 97 kb). The major 21.4 kb duplication class that appears in most lanes corresponds to the configuration where a full rDNA repeat (9.2 kb) separates the duplicated inserts (6 kb of the *Hin*dIII rDNA fragment, plus 1.8 kb of the *Bam*HI *HIS3* fragment and 4.4 kb of pBR322). (**B**) Chromosomal DNA from two independent *top1* sectored colonies [17 and 18 in (A)] was partially digested with *Sal*I at 37°C according to the conditions shown above the figure. After digestion, the fragments were separated by CHEF electrophoresis for 20 h at 200 V with a pulse time ramping from 1 to 50 s and, after transfer to a nylon membrane, the blot was hybridized with $^{32}$P-labeled pUC18 DNA. Since the only signal detected corresponds to the length of one single unit between the inserts, this clearly shows that the marker duplication is located at a unique position in the tandem array. (**C**) DNA was analyzed as described in (A) except that electrophoresis pulse time ramping was from 1 to 10 s for 18 h at 200 V. DNA was isolated from: a wild-type strain with a duplicated marker in rDNA (lane 1), unsectored *top3-3*::*LEU2* rDNA::*URA3* colonies (lanes 2–5) and sectored *top3-3*::*LEU2* rDNA::*URA3* colonies (lanes 6–28). The membrane was hybridized with the 1.3 kb *Sac*I–*Aat*II $^{32}$P-labeled *lacZ* fragment. (**D**) A similar experiment to that described in (A) was performed with *top1-8*::*LEU2 rad52-8*::*TRP1* rDNA::*HIS3* segregants obtained from the same cross used to derive the segregants analyzed in (A). Lanes 1–6 contains DNA from unsectored *top1-8*::*LEU2 rad52-8*::*TRP1* rDNA::*HIS3* colonies and lanes 7–22 contain DNA from sectored *top1-8*::*LEU2 rad52-8*::*TRP1* rDNA::*HIS3* colonies. A single marker duplication event was observed (lane 7). (**E**) In this panel, sectored colonies from *top3-3*::*LEU2 rad52-8*::*TRP1* rDNA::*URA3* segregants obtained from the same cross used to derive the segregants shown in (C) were examined. The strains were analyzed as described in (A), except that the fragments were separated by CHEF electrophoresis for 15 h at 200 V with a pulse time ramping from 0.2 to 13 s. The membrane was hybridized with the same probe used in (C). A single marker duplication was observed (lane 20).

were observed amongst the six non-sectored colonies analyzed in the double mutants (Figure 5D, lanes 1–6). These results indicate that most duplications observed in *top1* cells require a functional *RAD52* pathway. Longer exposure of the autoradiogram shown in Figure 5D reveals that four of the 16 *top1 rad52* sectored colonies contain a few weak signals that correspond to secondary events (data not shown). This indicates that marker duplication can still occur in the absence of a functional *RAD52* gene. However, the number of repeats between the markers in these secondary events ranges from two to seven compared with those duplications generated in *top1* mutants, which are mainly separated by a single repeat.

The same approach described above for *top1* was used to analyze *top3* mutants. Similarly to *top1*, *top3* mutants exhibit an elevated level of duplications in both sectored (Figure 5C, lanes 6–28) and unsectored colonies (Figure 5C, lanes 2–5). Unlike that found in *top1* mutants, the duplications in *top3* cells are separated by a broader range of rDNA repeat lengths (from one to 20 or more). Although a major duplication class can be detected occasionally in a sectored colony (e.g. Figure 5C, lane 28), in most cases, multiple weaker signals are detected. Again, this is indicative of independent events that occur during the growth of the colony. In *top3 rad52* segregants, marker duplications, which were ladders in *top3* mutants, were barely detectable (Figure 5E).

Thus, in the absence of either of the two type I topoisomerases, yeast cells display different rearrangements in the rDNA tandem array. However, unlike the frequency of marker loss in *top1* and *top3* strains, which differ with respect to their dependence upon *RAD52*, marker duplications in both topoisomerase mutants equally require *RAD52* gene function.

## Discussion

### *Classical USCE is not a frequent event in rDNA*

Although many groups have proposed that USCE is the primary mechanism by which tandemly repeated genes maintain their homogeneity or adapt their copy number to the environment (Petes, 1980; Szostak and Wu, 1980), our results indicate that it is not the case. We found that <30% of the events generating marker loss could be due to USCE. Furthermore, we had developed previously a visual assay that allows the determination of marker copy number in diploid cells based on colony color. Using this assay, we found that, in diploids, even fewer marker loss events were accompanied by marker duplication (2.6%, H.Zou and R.Rothstein, unpublished results).

One of the hallmarks of classical USCE is that the number of repeats separating the marker from the borders (represented by L and R in Figure 2A) is identical in the parent and the recombinants. In the T16 strain, due to the close proximity of the marker to border sequences, we were able to measure the length of fragment L. Surprisingly, changes in the length of L concomitant with marker loss were observed. We ruled out the possibility that the changes in L were due to pre-existing events by developing a quarter-sector assay where the parental configuration could be verified. The analysis confirms that a single event, like gene conversion between non-aligned sister chromatids (i.e. USCGC, see Figure 6), is responsible for

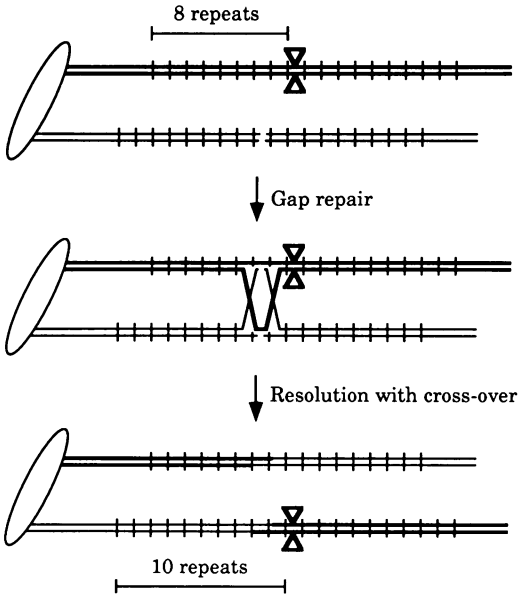


**Fig. 6.** A gene conversion model to explain some T16 recombinants. The marker inserted in rDNA is depicted by an inverted triangle located eight rDNA repeats away from unique border sequences. After replication, one of the $G_2$ sisters (represented by thin lines) contains a DSB in or close to the marker. This DSB is next converted to a larger gap that removes the marker sequence. The gap is repaired subsequently as described in the DSB repair model (Szostak *et al.*, 1983) by converting misaligned information from the sister chromatid (bold lines). Final resolution of this structure by a crossover generates the recombinant molecules. One chromatid has lost the marker while the other contains a single copy of the marker shifted two additional rDNA repeats from the unique border sequence.

the marker configuration described. Additionally, a novel class of recombinants was recovered where marker loss on one chromatid is associated with a marker duplication on the sister chromatid and at the same time is accompanied by a gain in the size of fragment L (see Figure 2C, lane 1 and Figure 3B, pair 7). This class of recombinant is best explained by two concerted conversion events similar to that described previously (Ray *et al.*, 1989).

### *Gene conversion can account for the majority of recombination events in rDNA*

*RAD52* plays an important role in mitotic and meiotic gene conversion (Malone and Esposito, 1980; Jackson and Fink, 1981; Klein, 1988; Bailis and Rothstein, 1990; Petes *et al.*, 1991). In its absence, there is a 10-fold increase in marker loss in rDNA. We explain this elevated recombination by the absence of gene conversion in *rad52* mutant cells. In wild-type cells, the *RAD52*-dependent pathway would most often repair faithfully spontaneous lesions by gene conversion without marker loss. However, in *rad52* mutant cells, repair by gene conversion would be blocked, forcing the same lesions to be repaired at the expense of adjacent repeats by a non-conservative SSA pathway (Ozenberger and Roeder, 1991). Thus, in rDNA, a lesion at or near the inserted marker would always result in marker loss via degradation necessary to uncover flanking homology and complete the SSA process. Our results support this view, since we found that the length of the rDNA array only shrinks in *rad52* mutants (Figure 4B). In contrast, both expansion and contraction of the

array is observed in wild-type cells where gene conversion functions (Figure 4A).

Our experiments with *top1* and *top3* mutants also support a role for gene conversion in the maintenance of stability of the rDNA array. The physical analysis presented here reveals that, in the absence of either *TOP1* or *TOP3*, marker duplications are greatly increased (Figure 5A and C). A marker duplication can be generated by gene conversion when a lesion in one or more rDNA repeat units is processed into a DSB and/or a gap. Subsequent repair of the break or gap from rDNA repeats containing the marker results in its duplication. In both *rad52 top1* and *rad52 top3* double mutants, the frequency of these duplications is greatly reduced. The dependence upon *RAD52* suggests that these duplications are the result of gene conversion.

### The pattern of marker duplications differs between top1 and top3 mutants

The marker duplications that occur spontaneously in *top1* and *top3* mutants exhibit different patterns. For *top1* mutants, the markers are separated by one rDNA repeat (Figure 5A), while in *top3* mutants, the duplications are separated by a wide range of repeat units (from 0 to >20) (Figure 5C). For *top1*, we showed that there is a single marker duplication per chromosome (Figure 5B). Due to its dispersed pattern, a similar analysis could not be performed for *top3* mutants. The difference in duplication patterns between these strains may be a consequence of the structure of the nucleolus and the organization of the rDNA. A model has been proposed in which the enhancers and promoters of each repeat are anchored to the nucleolar matrix, resulting in a structure where each rDNA repeat forms a loop (Johnson and Warner, 1989; Kulkens *et al.*, 1992). We suggest that, in the absence of *TOP1*, gene conversion may be restricted to adjacent loops. For example, a lesion in the repeat adjacent to the marker may be repaired preferentially from the neighboring marker-containing loop, resulting in one rDNA unit separating the two markers. However, additional studies are necessary to determine the cause of the high frequency of initial marker duplication and the subsequent failure to observe the expansion of the marker across the rDNA array.

The duplication pattern observed in *top3* mutants is more disperse. Previously, we suggested that, in the absence of *TOP3*, newly replicated molecules remain catenated and undergo breaks during chromosome separation (Gangloff *et al.*, 1994a). The unequal repair of these breaks by gene conversion may redistribute the marker across the array. Repetition of this process would result in the rapid appearance of multiple bands that we detect in some *top3* clones (e.g. lanes 17 and 19 in Figure 5C).

### RAD52 is required differentially for recombination in topoisomerase mutants

The Top1 and Top3 enzymes display a differential requirement for *RAD52* only with respect to marker loss (Table I). The postulated biochemical activities of these enzymes may explain this differential requirement. For example, Top1 is involved primarily in the relaxation of supercoils generated by polymerase I transcription (Brill *et al.*, 1987; Brill and Sternglanz, 1988) and also acts as a swivel for transcription and replication throughout the cell cycle (Brill *et al.*, 1987; Kim and Wang, 1989). Top3 probably plays a role in the decatenation of single-stranded molecules in the late $S/G_2$ phase to disentangle replicating chromatids (Wang, 1991; Gangloff *et al.*, 1994a). Lesions that result from the absence of these enzymes may generate different substrates for repair. Alternatively, the differential requirement for *RAD52* may reflect the occurrence of lesions at different phases of the cell cycle when the enzymes normally act. These hypotheses are not mutually exclusive. For example, our results may be explained by a combination of the two models where both the nature of the lesions and the timing of their occurrence contribute to the differential effects.

### Gene conversion as a universal mechanism for sequence expansion and correction

Here, we have shown that USCE is not the major recombination mechanism operating in the rDNA multiple tandem array. We propose that most of the alterations in this array are the result of gene conversion events. Rigorous demonstration that the events are truly gene conversion (i.e. non-reciprocal) requires accurate measurement of the length of the array in both products of a single event. Due to its large size (1.5–2.0 Mb), it is impossible to measure directly small variations in the length of the array before and after an event. However, several lines of evidence support the view that gene conversion is the mechanism responsible for most of the alterations we observe. First, the generation of duplications and the loss of markers that are greatly stimulated in the absence of *TOP1* and *TOP3* require *RAD52* function, a gene product necessary for gene conversion (Petes *et al.*, 1991). Secondly, the distinct loss of rDNA repeats in *rad52* mutants is probably due to SSA, which is likely to be the default pathway when gene conversion is blocked. Finally, 6/21 marker loss events in the T16 strain are most easily interpreted as the result of gene conversion (see Figure 6). In fact, this number is an underestimate since we can only detect changes in L with current methods. Due to the parity of gene conversion (Fogel *et al.*, 1981), approximately half of the initiated events result in a change in R, which we cannot detect.

Conversion events similar to the ones described here are not restricted to mitosis. Fogel and his co-workers found that a multiple tandem array at the *CUP1* locus could undergo contractions and expansions during meiosis (Welch *et al.*, 1990). We suggest that those events are generated by gene conversion similarly to the ones we describe at rDNA. DSBs initiated during meiosis are probably the lesions responsible for the *CUP1* events. The repair of these initiating lesions from a non-aligned copy (or copies) of the repeat using either the homolog or a sister chromatid could generate the gains or losses they observed.

Recently, Vogt and his colleagues introduced an endonuclease from *Physarum polycephalum* into yeast, that recognizes a unique site present only in each rDNA repeat (Muscarella and Vogt, 1993). Induction of the enzyme is lethal to most of the cells but, at a frequency of $\sim 4 \times 10^{-3}$, colonies arise that only contain mutated copies of the rDNA that are resistant to digestion by the enzyme. They suggested several mechanisms to explain this event. We favor the interpretation that, in the population of cells,

there is a pre-existing mutation in one or a few repeats. We suggest that the introduction of the DSBs by the endonuclease triggers a checkpoint (Weinert and Hartwell, 1993) that arrests the cells until all the lesions are repaired by gene conversion. The digested repeats can only be repaired using the uncut sequence(s). Since the ectopically expressed endonuclease is continually present, the only cells that will survive are those that have replaced the wild-type repeats with the uncuttable units. Therefore, multiple rounds of gene conversion allow the mutation to spread rapidly throughout the array, giving rise to a completely resistant yeast cell.

Gene conversion may also play a role in homogenizing multiple tandem arrays in mammalian cells. Roberts and Axel (1982) observed high frequency gene correction within an amplified array in mammalian somatic cells. The amplified array contains 20–40 copies of *aprt,* which was co-amplified by selecting for function of an adjacent promoterless *tk* gene. At a remarkably high frequency ($\sim 10^{-4}$), they found aprt$^-$ tk$^+$ clones in which all of the copies of *aprt* contained the identical alteration. Individual aprt$^-$ clones contained different mutations and, in most cases, the *aprt* gene was either deleted or its transcription was decreased dramatically. It is well known that transcription stimulates recombination (for review, see Gangloff *et al.*, 1994b) and, in both yeast and chickens, transcriptionally active regions are preferential recipients during gene conversion (Klar *et al.*, 1981; Nasmyth *et al.*, 1981; McCormack and Thompson, 1990). Thus, we propose that, once a transcriptionally inactive allele arises in one copy of the array, it would be used as the preferred donor of genetic information to repair lesions stimulated by transcription of the active copies. Fixation of the mutation would occur similarly to that described above for the *Physarum* endonuclease-stimulated events in yeast.

Finally, our studies may provide an explanation for the expansion of triplet repeats associated with many neurological diseases such as fragile X (Fu *et al.*, 1991), myotonic dystrophy (Fu *et al.*, 1992; Harley *et al.*, 1992), spinal and bulbar muscular atrophy (La Spada *et al.*, 1991; Caskey *et al.*, 1992) and Huntington's disease (Huntington's Disease Collaborative Research Group, 1993). In the case of *FMR-1* and *MT-PK* (for review, see Kuhl and Caskey, 1993), most alleles in the population contain between six and 54 triplet repeats. A subset of individuals exists in a pre-mutation state, where the alleles contain 50–200 triplets. Individuals carrying these alleles can give rise to affected offspring whose triplet repeat number can be as great as 4000. It is significant that the rapid expansion is not observed until a minimum repeat length is reached: 150–600 nucleotides. This may relate to a minimum size requirement for pairing during homologous recombination (Liskay and Stachelek, 1986; Jinks-Robertson *et al.*, 1993). As no single meiotic event can generate such an increase, we propose that mitotic events in the divisions preceding germ cell formation may create these expanded arrays via a gene conversion process similar to the one just described. Furthermore, mutations that increase mitotic recombination (like *top1* or *top3*) may, in fact, predispose some carriers of these pre-mutations to expand their repeats more rapidly than others.

## Materials and methods

### *Yeast strains and genetic methods*

All of the strains used in this study to measure recombination in rDNA are isogenic haploid derivatives of W303-*MAT***a** or *MAT*α *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100* (Thomas and Rothstein, 1989). The *top1-8*::*LEU2* mutant was kindly provided by Rolf Sternglanz (Brill and Sternglanz, 1988). The *top3-2*Δ::*HIS3* allele (Gangloff *et al.*, 1994a) was constructed by transforming W303 using pWJ201 (Wallis *et al.*, 1989). The *top3-3*Δ::*LEU2* allele (Gangloff *et al.*, 1994a) was constructed by combining two extreme transposon insertions (Seifert *et al.*, 1986) that functionally inactivate *TOP3* (Wallis and Rothstein, unpublished observations). This creates a deletion that retains a functional *LEU2* gene in place of most of the coding sequence. The *rad52-8*::*TRP1* allele was introduced into the W303 background using a disruption plasmid provided by David Schild (Schild *et al.*, 1983). W979-3B, *MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100* rDNA::*URA3* and W878-1D, *MAT***a** *leu2-3,112 his3-11,15 ura3-1 ade2-1 trp1-1 can1-100* rDNA::*HIS3* are W303 derivatives of the rDNA insertion strains (CY143 and CY147) constructed and provided by M.Christman (Christman *et al.*, 1988). The *top2-4* allele was introduced into the W303 background using pCH510 kindly provided by Connie Holm. The T16 strain (Szostak and Wu, 1980) was kindly provided by Louise Prakash. Standard yeast genetic methods were used for the analysis of strains and crosses (Sherman *et al.*, 1986).

### *Determination of the recombination frequencies in rDNA*

Since we are analyzing recombination in known hyper-rec mutants, the frequency of marker loss events is dependent upon the original marker arrangement and number. To minimize the extent of marker rearrangements that could arise during growth of the culture, two precautions were taken. First, wild-type reference strains were isolated that contain a single copy of the marker at a known position. CHEF gel analysis revealed that ~50 rDNA repeats for *HIS3* (W878-1D) and ~80 repeats for *URA3 (*W979-3B) separate these markers from the closest unique sequences (data not shown). Secondly, the hyper-rec mutants were crossed to these reference strains to form diploids that are heterozygous for both the marked rDNA and the hyper-rec mutation. Since the hyper-rec mutations are recessive, this ensures that the position of the marker within the rDNA array is maintained during strain construction with the same stability as in wild-type. Spore clones containing the relevant genotypes were generated from these diploids and were grown for <20 generations before measuring recombination frequencies. Cells were analyzed immediately by resuspension in water and plating onto YPD. After growth, the colonies were replica plated to the appropriate omission medium. Recombination frequencies were determined by counting the number of colonies that failed to grow and dividing by the total number of cells plated.

### *Preparation of intact chromosomal DNA and pulsed-field gel electrophoresis*

Chromosomal DNA was embedded into agarose blocks using the method described by Gerring *et al.* (1991). The blocks were digested with *Sal*I or *Pst*I according to the protocol provided by the manufacturer (New England Biolabs). CHEF gels (1% agarose) were all run in a Bio-Rad CHEF-DR® II System at 14°C in 0.5× TBE buffer.

### *Genomic blots and hybridization procedures*

Genomic and chromosome blots were carried out on Hybond™-N+ according to the alkaline technique described by the manufacturer (Amersham). Random priming was used to label the probes with [$\alpha$-$^{32}$P]dCTP, and hybridizations were performed according to standard procedures (Sambrook *et al.*, 1989) in a Hybaid oven. To minimize cross-hybridization with the endogenous *his3* or *ura3* genes, vector sequences (pUC18, pBR322 or *lacZ*) were labeled as probes to detect the various duplications. To minimize contamination of the rDNA probe, a nested PCR strategy was used. A first round of colony PCR (Huxley *et al.*, 1990) was performed using the primers 5′-AACCAGCAAATG-CTAGCACCAC-3′ and 5′-CCTAATTCAGGGAGGTAGTGAC-3′ to generate a 913 bp product within the 18S RNA coding region. A second round of PCR was performed on a 1000-fold dilution of this template using the primers 5′-GGAGGGCAAGTCTGGTGCCAGC-3′ and 5′-CGGCCATGCACCACCACCCACA-3′ to generate a 723 bp rDNA probe.

### *Statistical analyses*

All statistical analyses were performed with Statview 4.0 software (Abacus Concepts), using the 2×2 contingency test with correction or the paired *t*-test.

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## References

Adzuma,K., Ogawa,T. and Ogawa,H. (1984) Primary structure of the *RAD52* gene in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **4**, 2735–2744.

Bailis,A.M. and Rothstein,R. (1990) A defect in mismatch repair in *Saccharomyces cerevisiae* stimulates ectopic recombination between homologous genes by an excision repair dependent process. *Genetics*, **126**, 535–547.

Bendixen,C., Sunjevaric,I., Bauchwitz,R. and Rothstein,R. (1994) Identification of a mouse homologue of the *Saccharomyces cerevisiae* recombination and repair gene, *RAD52*. *Genomics*, **23**, 300–303.

Bezzubova,O.Y., Schmidt,H., Ostermann,K., Heyer,W.D. and Buerstedde,J.M. (1993) Identification of a chicken *RAD52* homologue suggests conservation of the *RAD52* recombination pathway throughout the evolution of higher eukaryotes. *Nucleic Acids Res.*, **21**, 5945–5949.

Brill,S.J. and Sternglanz,R. (1988) Transcription-dependent DNA supercoiling in yeast DNA topoisomerase mutants. *Cell*, **54**, 403–411.

Brill,S.J., DiNardo,S., Voelkel-Meiman,K. and Sternglanz,R. (1987) Need for DNA topoisomerase activity as a swivel for DNA replication for transcription of ribosomal RNA. *Nature*, **326**, 414–416.

Caskey,C.T., Pizzuti,A., Fu,Y.H., Fenwick,R.G.,Jr and Nelson,D.L. (1992) Triplet repeat mutations in human disease. *Science*, **256**, 784–789.

Charlesworth,B., Sniegowski,P. and Stephan,W. (1994) The evolutionary dynamics of repetitive DNA in eukaryotes. *Nature*, **371**, 215–220.

Christman,M.F., Dietrich,F.S. and Fink,G.R. (1988) Mitotic recombination in the rDNA of *Saccharomyces cerevisiae* is suppressed by the combined action of DNA topoisomerases I and II. *Cell*, **55**, 413–425.

Edelman,G.M. and Gally,J.A. (1970) *Arrangement and Evolution of Eukaryotic Genes*. Rockefeller University Press, New York.

Fogel,S., Mortimer,R.K. and Lusnak,K. (1981) *Mechanisms of Meiotic Gene Conversion, or 'Wanderings on a Foreign Strand'*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Fu,Y.H., Kuhl,D.P., Pizzuti,A., Pieretti,M., Sutcliffe,J.S., Richards,S., Verkerk,A.J., Holden,J.J., Fenwick,R.Jr, Warren,S.T. *et al.* (1991) Variation of the CGG repeat at the fragile X site results in genetic instability: resolution of the Sherman paradox. *Cell*, **67**, 1047–1058.

Fu,Y.H., Pizzuti,A., Fenwick,R.Jr, King,J., Rajnarayan,S., Dunne,P.W., Dubel,J., Nasser,G.A., Ashizawa,T., de Jong,P. *et al.* (1992) An unstable triplet repeat in a gene related to myotonic muscular dystrophy. *Science*, **255**, 1256–1258.

Gangloff,S., McDonald,J.P., Bendixen,C., Arthur,L. and Rothstein,R. (1994a) The yeast type I topoisomerase Top3 interacts with Sgs1, a DNA helicase homolog: a potential eukaryotic reverse gyrase. *Mol. Cell. Biol.*, **14**, 8391–8398.

Gangloff,S., Lieber,M.R. and Rothstein,R. (1994b) Transcription, topoisomerases and recombination. *Experientia*, **50**, 261–269.

Gerring,S.L., Connelly,C. and Hieter,P. (1991) Positional mapping of genes by chromosome blotting and chromosome fragmentation. In Guthrie,C. and Fink,G.R. (eds), *Positional Mapping of Genes by Chromosome Blotting and Chromosome Fragmentation*. Academic Press Inc., San Diego, CA, Vol. 194, pp. 57–77.

Harley,H.G., Brook,J.D., Rundle,S.A., Crow,S., Reardon,W., Buckler,A.J., Harper,P.S., Housman,D.E. and Shaw,D.J. (1992) Expansion of an unstable DNA region and phenotypic variation in myotonic dystrophy. *Nature*, **355**, 545–546.

Huntington's Disease Collaborative Research Group (1993) A novel gene containing a trinucleotide repeat that is expanded and unstable on Huntington's disease chromosomes. *Cell*, **72**, 971–983.

Huxley,C., Green,E.D. and Dunham,I. (1990) Rapid assessment of *S.cerevisiae* mating type by PCR. *Trends Genet.*, **6**, 236.

Jackson,J.A. and Fink,G.R. (1981) Gene conversion between duplicated genetic elements in yeast. *Nature*, **292**, 306–311.

Jinks-Robertson,S., Michelitch,M. and Ramcharan,S. (1993) Substrate length requirements for efficient mitotic recombination in *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **13**, 3937–3950.

Johnson,S.P. and Warner,J.R. (1989) Unusual enhancer function in yeast rRNA transcription. *Mol. Cell. Biol.*, **9**, 4986–4993.

Kim,R.A. and Wang,J.C. (1989) Function of DNA topoisomerases as replication swivels in *Saccharomyces cerevisiae*. *J. Mol. Biol.*, **208**, 257–267.

Klar,A.J., Strathern,J.N. and Hicks,J.B. (1981) A position-effect control for gene transposition: state of expression of yeast mating-type genes affects their ability to switch. *Cell*, **25**, 517–524.

Klein,H.L. (1984) Lack of association between intrachromosomal gene conversion and reciprocal exchange. *Nature*, **310**, 748–753.

Klein,H.L. (1988) Different types of recombination events are controlled by the *RAD1* and *RAD52* genes of *Saccharomyces cerevisiae*. *Genetics*, **120**, 367–377.

Klein,H.L. and Petes,T.D. (1981) Intrachromosomal gene conversion in yeast. *Nature*, **289**, 144–148.

Kuhl,D.P. and Caskey,C.T. (1993) Trinucleotide repeats and genome variation. *Curr. Opin. Genet. Dev.*, **3**, 404–407.

Kulkens,T., van der Sande,C.A., Dekker,A.F., van-Heerikhuizen,H. and Planta,R.J. (1992) A system to study transcription by yeast RNA polymerase I within the chromosomal context: functional analysis of the ribosomal DNA enhancer and the RBP1/REB1 binding sites. *EMBO J.*, **11**, 4665–4674.

La Spada,A.R., Wilson,E.M., Lubahn,D.B., Harding,A.E. and Fischbeck,K.H. (1991) Androgen receptor gene mutations in X-linked spinal and bulbar muscular atrophy. *Nature*, **352**, 77–79.

Larionov,V.L., Grishin,A.V. and Smirnov,M.N. (1980) 3 μm DNA—an extrachromosomal ribosomal DNA in the yeast *Saccharomyces cerevisiae*. *Gene*, **12**, 41–49.

Lehrman,M.A., Goldstein,J.L., Russell,D.W. and Brown,M.S. (1987) Duplication of seven exons in LDL receptor gene caused by Alu–Alu recombination in a subject with familial hypercholesterolemia. *Cell*, **48**, 827–835.

Levin,N.A., Bjornsti,M.A. and Fink,G.R. (1993) A novel mutation in DNA topoisomerase I of yeast causes DNA damage and *RAD9*-dependent cell cycle arrest. *Genetics*, **133**, 799–814.

Liskay,R.M. and Stachelek,J.L. (1986) Information transfer between duplicated chromosomal sequences in mammalian cells involves contiguous regions of DNA. *Proc. Natl Acad. Sci. USA*, **83**, 1802–1806.

Malone,R. and Esposito,R.E. (1980) The *RAD52* gene is required for homothallic interconversion of mating types and spontaneous mitotic recombination in yeast. *Proc. Natl Acad. Sci. USA*, **77**, 503–507.

McCormack,W.T. and Thompson,C.B. (1990) Chicken IgL variable region gene conversions display pseudogene donor preference and 5′ to 3′ polarity. *Genes Dev.*, **4**, 548–558.

Muris,D.F., Bezzubova,O., Buerstedde,J.M., Vreeken,K., Balajee,A.S., Osgood,C.J., Troelstra,C., Hoeijmakers,J.H., Ostermann,K., Schmidt,H. *et al.* (1994) Cloning of human and mouse genes homologous to *RAD52*, a yeast gene involved in DNA repair and recombination. *Mutat. Res.*, **315**, 295–305.

Muscarella,D.E. and Vogt,V.M. (1993) A mobile group I intron from *Physarum polycephalum* can insert itself and induce point mutations in the nuclear ribosomal DNA of *Saccharomyces cerevisiae*. *Mol. Cell. Biol.*, **13**, 1023–1033.

Nasmyth,K.A., Tatchell,K., Hall,B.D., Astell,C. and Smith,M. (1981) Physical analysis of mating-type loci in *Saccharomyces cerevisiae*. *Cold Spring Harbor Symp. Quant. Biol.*, **45**, 961–981.

Oberle,I., Rousseau,F., Heitz,D., Kretz,C., Devys,D., Hanauer,A., Boue,J., Bertheas,M.F. and Mandel,J.L. (1991) Instability of a 550-base pair DNA segment and abnormal methylation in fragile X syndrome. *Science*, **252**, 1097–1102.

Ostermann,K., Lorentz,A. and Schmidt,H. (1993) The fission yeast *rad22* gene, having a function in mating-type switching and repair of DNA damages, encodes a protein homolog to Rad52 of *Saccharomyces cerevisiae*. *Nucleic Acids Res.*, **21**, 5940–5944.

Ozenberger,B.A. and Roeder,G.S. (1991) A unique pathway of double-strand break repair operates in tandemly repeated genes. *Mol. Cell. Biol.*, **11**, 1222–1231.

Petes,T.D. (1979) Yeast ribosomal DNA genes are located on chromosome XII. *Proc. Natl Acad. Sci. USA*, **76**, 410–414.

Petes,T.D. (1980) Unequal meiotic recombination within tandem arrays of yeast ribosomal DNA genes. *Cell*, **19**, 765–774.
Petes,T.D., Malone,R.E. and Symington,L.S. (1991) *Recombination in Yeast*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Ray,A., Machin,N. and Stahl,F.W. (1989) A DNA double chain break stimulates triparental recombination in *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **86**, 6225–6229
Resnick,M.A. (1969) Genetic control of radiation sensitivity in *Saccharomyces cerevisiae*. *Genetics*, **62**, 519–531.
Roberts,J.M. and Axel,R. (1982) Gene amplification and gene correction in somatic cells. *Cell*, **29**, 109–119.
Sambrook,J., Fritsch,E.F. and Maniatis,T. (1989) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Schild,D., Calderon,I.L., Contopoulou,R. and Mortimer,R.K. (1983) Cloning of yeast recombination repair genes and evidence that several are non-essential genes. In Bridges,B.A. and Friedberg,E.C. (eds), *Cloning of Yeast Recombination Repair Genes and Evidence that Several are Non-essential Genes*. A.R.Liss, New York, pp. 417–427.
Seifert,H.S., Chen,E.Y., So,M. and Heffron,F. (1986) Shuttle mutagenesis: a method of transposon mutagenesis for *Saccharomyces cerevisiae*. *Proc. Natl Acad. Sci. USA*, **83**, 735–739.
Sherman,F., Fink,G.R. and Hicks,J.B. (1986) *Methods in Yeast Genetics*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Szostak,J.W. and Wu,R. (1980) Unequal crossing over in the ribosomal DNA of *Saccharomyces cerevisiae*. *Nature*, **284**, 426–430.
Szostak,J.W., Orr-Weaver,T.L., Rothstein,R.J. and Stahl,F.W. (1983) The double-strand-break repair model for recombination. *Cell*, **33**, 25–35.
Thomas,B.J. and Rothstein,R. (1989) Elevated recombination rates in transcriptionally active DNA. *Cell*, **56**, 619–630.
Wallis,J.W., Chrebet,G., Brodsky,G., Rolfe,M. and Rothstein,R. (1989) A hyper-recombination mutation in *S.cerevisiae* identifies a novel eukaryotic topoisomerase. *Cell*, **58**, 409–419.
Wang,J.C. (1991) DNA topoisomerases: why so many? *J. Biol. Chem.*, **266**, 6659–6662.
Warburton,P.E., Waye,J.S. and Willard,H.F. (1993) Nonrandom localization of recombination events in human alpha satellite repeat unit variants: implications for higher-order structural characteristics within centromeric heterochromatin. *Mol. Cell. Biol.*, **13**, 6520–6529.
Weinert,T.A. and Hartwell,L.H. (1993) Cell cycle arrest of *cdc* mutants and specificity of the *RAD9* checkpoint. *Genetics*, **134**, 63–80.
Welch,J.W., Maloney,D.H. and Fogel,S. (1990) Unequal crossing-over and gene conversion at the amplified *CUP1* locus of yeast. *Mol. Gen. Genet.*, **222**, 304–310.
Willis,K.K. and Klein,H.L. (1987) Intrachromosomal recombination in *Saccharomyces cerevisiae*: reciprocal exchange in an inverted repeat and associated gene conversion. *Genetics*, **117**, 633–643.
Zamb,T.J. and Petes,T.D. (1981) Unequal sister-strand recombination within yeast ribosomal DNA does not require the *RAD52* gene product. *Curr. Genet.*, **3**, 125–132.

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